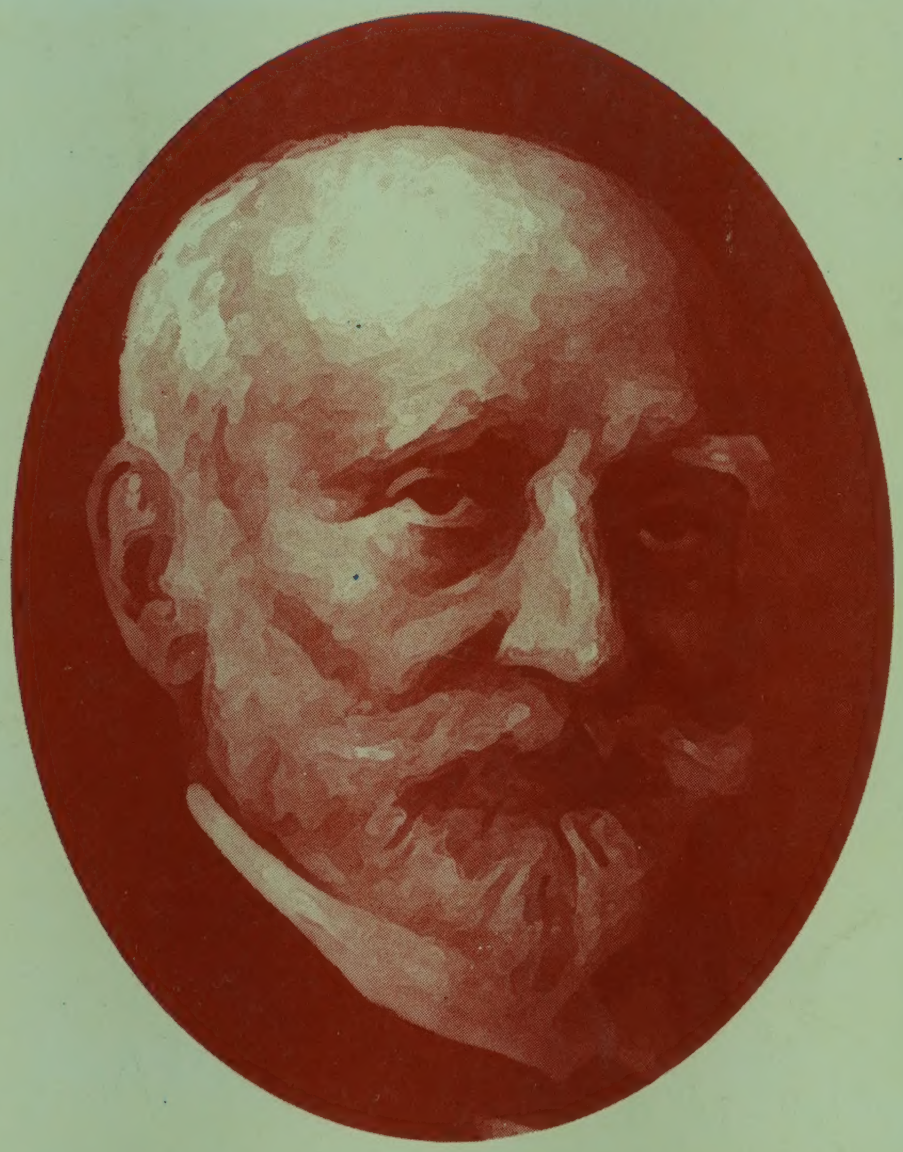
The Second Sir Dorabji Tata Symposium

on

Malaria : The Indian Scene
&
Vaccine Research in India



Sir Dorabji Tata Centre for Research in Tropical Diseases

&

Department of Microbiology and Cell Biology

Indian Institute of Science

Bangalore - 560 012, India

The Second Sir Dorabji Tata Symposium

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Malaria: The Indian Scene
&
Vaccine Research in India

March 10-11, 2001

Programme & Abstracts

Sir Dorabji Tata Centre for Research
in Tropical Diseases
&
Department of Microbiology and Cell Biology
Indian Institute of Science,
Bangalore - 560012, India

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Foreword

It is a pleasure to present the Second Sir Dorabji Tata symposium to the scientific community. It is the first one after the formal inauguration of the Sir Dorabji Tata Centre for Research in Tropical Diseases (SDTC) on 26th May 2000.

SDTC is the result of the vision of Sir Dorabji Tata, the second head of the Tata business ventures. He had envisaged this institution to develop in parallel with the Indian Institute of Science (IISc). While IISc took off and attained a premier status, SDTC was a non-starter due to various reasons. The idea was revived in 1998 when it was realised that science should have a human face. The Memorandum of Understanding that established the centre envisages such a link between science laboratories and the medical profession. Amongst the activities of the Centre that devolve from the mandate the holding of symposia on important topics would be an important one.

The first symposium was held on 11th and 12th March 2000. It focused on the most important infection challenging India- ***Tuberculosis***. The dates were chosen to coincide with the date of establishment of the Sir Dorabji Tata Trust. This second symposium is likewise scheduled to include 11th March and address two important topics, Malaria and Vaccinology.

Malaria remains a major scourge in our country and crops up whenever a conducive environment develops. Additionally, large areas of the country are still endemic for the infection. The infection has demonstrated an ability to counter the various control measures. In this symposium we are looking at the recent observations in various aspects and would probably throw up ideas that may break the vicious cycle. Can we have a viable vaccine as an answer?

Ever since Jenner demonstrated the efficacy of the smallpox vaccine, the modality has been applied to a number of infections with varying success. With specific drugs and antibiotics becoming progressively costly and ineffective due to pathogen adaptation, immunization offers a way out. Some of the newer vaccines like the ***naked*** DNA vaccines are showing promise that may yet help us in the war against infections. A wide variety is being presented here.

This symposium is the result of a willing and tireless cooperation between the biology departments of IISc and SDTC. SDTC is yet a fledgling organization and I am sure this effort can be improved considerably. I do hope you like the material presented, we promise to improve by and by.

Lt. Gen. D. Raghunath
Principal Executive

SCIENTIFIC PROGRAMME

VENUE:
J.N. TATA AUDITORIUM
INDIAN INSTITUTE OF SCIENCE CAMPUS
BANGALORE - 560012.

MARCH 10, 2001

MALARIA: THE INDIAN SCENE

09.00 - 09.15 a.m. **Inauguration**

09.15 – 09.45 a.m. **Keynote Address** (Malaria: An Indian Perspective)
(*Dr. V. Ramalingaswami*)

Session I: Current Medical Practice

Chairperson: Dr. (Lt. Gen.) N. R. Krishnan

09.45 - 10.15 a.m. Spectrum of Clinical Features of Malaria **(S-1)**
Lt. Col. A. S. Kashyap, AFMC, Pune.

10.15 - 10.45 Malaria in Primitive Tribal Population **(S-2)**
Dr. Neeru Singh, MRC Fd. Stn., Jabalpur

10. 45 - 11.15 Treatment Practices and Traditional Control Measures **(S-3)**
Dr. Shiv Lal, DGHS, New Delhi. / Dr G.P.S. Dhillon

11.15 - 11.45 Chemoprophylaxis of Malaria **(S-4)**
AVM S. K. Dham, Comd Hosp A F, Bangalore.

11.45 - 12.00 **TEA**

Session II: The Malaria Parasites and Vectors.

Chairperson: Dr. P. Balaram

12.00 - 12.20 Erythrocyte Invasion and Cytoadherence by Malaria Parasites **(S-5)**
Dr. Chetan Chitnis, ICGEB, New Delhi

12.20 - 12.40 Properties of Vectors Affecting Malaria Transmission **(S-6)**
Dr. Sarala Subbarao, MRC, Delhi.

12.40 - 13.00 The Use of Genetics in Vector Control **(S-7)**
Dr. N. J. Shetty, Bangalore University, Bangalore.

13.00 - 13.20 Molecular Biology of the Malarial Vector Mosquito **(S-8)**
Dr. D. Deobagkar, University of Pune, Pune.

13.20 - 14.10 LUNCH

Session III: Malaria Vaccine

Chairperson: Dr. G. Padmanaban

14.10 - 14.40 Immune Mechanisms in Malaria: Role of Vaccine in Control **(S-14)**
Dr. V. S. Chauhan, ICGEB, New Delhi.

14.40 - 15.10 Novel Target Proteins of *P. falciparum* **(S-13)**
Dr. Shobhona Sharma, TIFR, Mumbai.

15.10 - 15.30 TEA

Session IV: Drugs for Malaria

Chairpersons: Dr. A. Surolia and Dr. D. Raghunath

15.30 – 16.00 Drug Resistant Malaria **(S-9)**
Dr. Nilima Kshirsagar, TNMC and Nair Hospital, Mumbai.

16.00 - 16.30 Experimental Malaria Models: Application to Drug Research **(S-10)**
Dr. S. K. Puri, CDRI, Lucknow.

16.30 - 17.00 Future Therapeutic Targets to Treat Malaria **(S-11)**
Dr. G. Padmanaban, IISc, Bangalore.

17.00 - 17.30 Genomics and Drug Development in Malaria **(S-12)**
Dr. Namita Surolia, JNC, Bangalore.

17.30 – 17.45 TEA

Session V: The Social Paradigm in Malaria Research

Chairperson: To be announced

17.45 - 18.15 Beyond Medicine: The Challenge of Socio-Epidemiology **(S-15)**
Dr. Ravi Narayanan, Sochara, Bangalore.

18.15 - 18.45 Control of Malaria Transmission Using Larvivorus Fishes **(S-16)**
Dr. S. K. Ghosh, MRC Field Unit, Bangalore.

19.30 DINNER

MARCH 11, 2001

VACCINE RESEARCH IN INDIA

09.00 – 09.30 **Keynote Address** (Vaccine Research in India)
Dr. N. K. Ganguly, DG, ICMR, New Delhi.

Session VI: Enteric Diseases
Chairperson: Dr. Ragni Macaden

09.30 - 10.00 The Development and Value of Cholera Vaccines **(S-17)**
Dr. G. B. Nair, ICDDR, Dhaka

10.00 - 10.30 Vaccine Strategies Against Rotavirus Diarrhoea **(S-18)**
Dr. C. Durga Rao, IISc, Bangalore

10.30 - 11.00 Epidemiology of Rotavirus infection in India **(S-19)**
Prof. M. K. Bhan, AIIMS, New Delhi.

11.00 - 11.15 **TEA**

Session VII: Viral Diseases
Chairperson: Dr. K. P. Gopinathan

11.15 - 11.45 Prospect of an Indian HIV-1 subtype C DNA Vaccine **(S-20)**
Dr. P. Seth, AIIMS, New Delhi

11.45 - 12.15 Recombinant Vaccines for Japanese Encephalitis **(S-21)**
Dr. S. Vrati, NII, New Delhi.

12.15 - 12.45 Protection Studies in Rhesus Monkeys Against Hepatitis E Virus (HEV) by Recombinant Protein Immunisation **(S-22)**
Dr. S. K. Panda, AIIMS, New Delhi.

12.45 - 13.15 DNA Vaccines **(S-23)**
Dr. P. N. Rangarajan, IISc, Bangalore.

13.15 - 14.00 **LUNCH**

Session VIII: Vaccine Production in India

Chairperson: Dr. N. K. Ganguly

14.00 - 14.30 Establishing a Commercial Vaccine Production Facility- The Serum Institute Experience **(S-24)**
Dr. S. V. Kapre, Serum Inst., Pune.

Session IX: Mycobacteria

Chairperson: Dr. R. Nayak

15.00 - 15.30 The Need for an Alternative to BCG **(S-25)**
Dr. C. N. Paramasivam, TRC, Chennai.

15.30 - 16.00 Immunology of Tuberculosis: Possible Approaches for a Vaccine
Dr. S. Vijaya, IISc, Bangalore. **(S-26)**

16.00 -16.30 Field Trial of Leprosy Vaccines **(S-27)**
Dr. M. D. Gupte, Inst. of Chennai.

16.30 - 17.00 TEA

17.00 Valedictory

Malaria: The Indian Scene

March 10, 2001

Sir Ronald Ross was born on May 13, 1857 in Almora, India. He received the Nobel Prize for Physiology or Medicine in 1902 for his work on malaria. His discovery of the malarial parasite in the gastrointestinal-tract of the *Anopheles* mosquito led to the realization that malaria was transmitted by *Anopheles,* and laid the foundation for combating the disease.

After graduating in medicine (1879), Ross entered the Indian Medical Service and served in the third Anglo-Burmese War (1885). On leave he studied bacteriology in London (1888-89) and then returned to India, where, prompted by Patrick Manson's guidance and assistance, he began (1892) a series of investigations on malaria. He discovered the presence of the malarial parasite within the *Anopheles* mosquito in 1897. Using birds that were sick with malaria, he was soon able to ascertain the entire life cycle of the malarial parasite, including its presence in the mosquito's salivary glands. He demonstrated that malaria is transmitted from infected birds to healthy ones by the bite of a mosquito, a finding that suggested the disease's mode of transmission to humans.

Ross returned to England in 1899 and joined the Liverpool School of Tropical Medicine and later the University of Liverpool. He was knighted in 1911. In 1912 he became physician for tropical diseases at King's College Hospital, London, and later director of the Ross Institute and Hospital for Tropical Diseases, founded in his honour. In addition to mathematical papers, poems, and fictional works, he wrote *The Prevention of Malaria* (1910). Sir Ross died on Sept. 16, 1932, in Putney Heath, London, England.

(Source: http://www.britannica.com/bcom/eb/article/8/0,5716,65788 +1+64138,00.html)

Nobel Prize in Physiology or Medicine 1902

(Presentation Speech by Professor the Count K. A. H. Mörner, Rector of the Royal Caroline Institute)

Your Majesty, Your Royal Highnesses, Ladies and Gentlemen.

Among the stipulations Alfred Nobel set forth in his will, on which the Nobel Foundation is based, that concerning the international character of the prizes occupies an important place. This proves not only his love of mankind and his wish that we should regard one another as brethren, but it is also a witness of his extensive and prescient views more especially concerning medical science and its advancement.

All the branches of medical science and their promotors in different countries have the same ultimate aim, that of gaining the most thorough knowledge possible both about the human body and the processes in it, as also about noxious influences and the means of their prevention. All medical workers unite in pursuing that aim and in doing so feel members of one great fellowship. Nevertheless, the different fields of medical science lie at such a distance one from another, that the individual worker on many occasions must look afar in the attempt to get a thorough view of the progress of the work.

With respect to diseases they are often of different kinds and import in divers regions of the world. For instance, malaria is nowadays of little importance here in Sweden, whereas it is a veritable scourge in other regions. For elucidating this question by an instance from a European country, it may be mentioned that in Italy of late the annual average of deaths by malaria has been about 15,000, and the yearly number of cases is calculated as about two millions. Still more overwhelming are the numbers from India. Of the British Army, amounting to about 178,000 men, close upon 76,000 men were admitted into hospital for malarial fever in the year 1897. In this single year the mortality from fever among the civil population in India amounted to a total of more than five millions. It is moreover a well-known fact, that malaria dominates so severely in vast territories that it causes the very greatest difficulties for the cultivation of countries which, but for the malaria, are specially favoured by Nature.

The question of the real nature of malaria, its origin, its manner of entering the organism, and the consequent question of the possibility of preventing this disease, are all of the greatest importance and have from remote ages occupied investigators, for a long time without success.

A very important discovery concerning malaria was made - now long ago, more than two decades - when Laveran, a French army surgeon, ascertained, that malaria is a parasitic disease, caused by a very low form of animal life, that he found in the blood of malarious patients. By this discovery the name of Laveran has for ever become renowned in the history of malaria.

Research about malaria in the last two decades has chiefly been based on Laveran's discovery. Science has thereby been enriched with many an important fact. We have gained knowledge of the different forms of the malarial parasite in blood. We have found, that it differs in the special forms of the disease. We have learned the relations between the parasite and the red blood corpuscles, in which it is chiefly to be found. We have furthermore been able to survey the manner in which it multiplies in the blood; the Italian investigator Golgi has in this respect revealed the remarkable fact that the periodicity of the malarial attacks depends on the appearance of new generations of the parasite in the blood. We have moreover found allied parasites in the blood of several mammals and birds.

The important question, previously mentioned, as to the possibility of the malarial parasite living outside the body, and its way of obtaining entrance into the blood remained unanswered. For some reasons, among others owing to various facts that were known concerning other parasites of an animal nature, it was supposed that the malarial parasite in some way leaves the blood so as to exist in some form in nature, probably as a parasite of some other being. As nothing indicated that the parasite was to be found in the secretions or excretions, the supposition lay near at hand, that suctorial insects would assist in carrying the parasite to a place, where it had to pass the aforementioned part of its life-cycle. Attention was therefore directed to the mosquito, which was thus supposed to spread the malarious infection. The importance of the mosquito in this respect has now been proved. In this case, as in several others, tradition anticipated science; it is even said, that negroes in the East-Africa use the same name for the mosquito and for malaria.

The mosquito theory of malaria was introduced to science by King no less than 18 years ago. The theory, however, remained a conjecture without other evidence than some suggestions given by epidemiological observations. The attempts made in Italy in the early nineties with the view of examining the theory experimentally, and, eventually, proving it to be true, gave results that seemed anything but encouraging; being far more likely to prevent the investigators from following this line.

A person we deem of great merit concerning the solution of the problem is the English investigator, Patrick Manson. It was a change in the appearance of the parasite, which was sometimes observed to occur, as the blood is shed, that Manson especially regarded as the first stage of its life outside the body. This phenomenon has afterwards been shown by the American pathologist Mac Callum to imply an act of reproduction of the parasite. Manson was moreover guided by his experience regarding another parasite of the blood, a little worm, filaria, the transference of which from one part of its life-cycle to another he had found effected by the mosquito, and more particularly by special species of the mosquito. By his views set forth on malaria, and by exciting expectation that the solution of the malaria problem was to be found in the direction he indicated, Manson gave an impulse to the further testing of the mosquito-theory and at last to its being established. Manson, who lived in England, had no opportunity of taking up the experimental work of the problem. The solution came from India.

It was an English army surgeon in India, Ronald Ross, who, impressed by Manson's induction, undertook the experimental testing of the matter. Critically arranging his experiments, he caused mosquitoes that were hatched from larvae in the laboratory, to bite malarious patients, and endeavoured to follow the parasite in the body of the mosquitoes. The results of the first two years' labour, although assiduous and scrupulous, gave little promise of success. But in August 1897 all at once he made vast progress towards his aim. While experimenting with another, less common species of mosquito, in the wall of its stomach he found bodies that very probably were an evolutionary stage of the human malarial parasite.

Ross, being prevented by circumstances from pursuing his plan in studying the malarial parasite of man, continued his work with an allied malarial parasite of birds. The result was that not only could he confirm his discovery concerning human malaria, as he found corresponding facts for avian malaria, but he also in a short time succeeded in revealing the further development of the avian malarial parasite in the body of the mosquito.

This development is briefly as follows. In the stomach of the mosquito a process of fecundation at first takes place; the form of the parasite, thereby produced, penetrates the stomach wall, embedded in which it grows to button-like structures projecting into the body-cavity. In these structures a large number of elongated organisms, sporozoites, are formed. On the consequent bursting of the said structures, the sporozoites escape into the general body-cavity of the mosquito, and accumulate in the salivary or poison glands, which are in connection with the proboscis with which the

bites of the insect are inflicted. A bite of the mosquito, at that time, inoculates the parasite, and if the individual is susceptible to the parasite, this develops in the manner known and described long ago.

Ross's discoveries into malaria were immediately followed by a series of important works.

Thus the Italian investigator, Grassi, in association with his colleagues, Bignami and Bastianelli, proved that the human malarial parasite not only in its early stage, already detected by Ross, but also in its further development undergoes the same evolution that Ross described for the growth of the avian malarial parasite in the body of the mosquito. Grassi also has precisely indicated the species of mosquito that are of import for the malaria of man. Many valuable works, besides these, have been issued by Ross, by the Italian investigators, by Robert Koch and by many others, works, by which not only our knowledge of the malarial parasite has been enlarged, but this knowledge has been made useful in combating and preventing malarial disease.

The eminent scientific value of Ross's work, its importance as a basis for the success of the recent investigations into malaria, its rich contents as regards the art of medical practice and especially hygiene, will be obvious from the above.

It is owing to these merits, that the Professorial Staff of the Royal Caroline Institute has decided to allot the Medical Nobel Prize of this year to Ronald Ross.

Professor Ronald Ross. In announcing that the Professorial Staff of the Royal Caroline Institute has decided to award to you the Medical Nobel Prize of this year on account of your work on malaria, in the name of the said Institute I congratulate you on your investigations. By your discoveries you have revealed the mysteries of malaria. You have enriched science with facts of great biological interest and of the very greatest medical importance. You have founded the work of preventing malaria, this veritable scourge of many countries.

(Source:http://www.nobel.se/medicine/laureates/1902/press.html; *Nobel Lectures*, Physiology or Medicine 1901-1921).

Abstracts

March 10, 2001

S-1

Spectrum of Clinical Features of Malaria

Col. S. R. Mehta VSM, Professor and HOD
Lt. Col. A. S. Kashyap, Reader
Dept of Medicine, AFMC Pune - 411040.

Malaria is going to remain a major problem all over the world. Manifestations of malaria mainly of *Plasmodium falciparum* have always been a diagnostic challenge even to the most astute clinician. The clinical presentation is also getting more and more atypical due to wide spread use of empirical treatment, drug resistance and other unknown factors. The delay in diagnosis, which is not infrequent even at best of centres, is related to atypical presentation and genuinely low peripheral parasitaemia. The low parasitaemia is due to most of the parasites remaining in deep visceral vessels because of the cytoadherent property of the parasitised red blood cells.

The net result of delayed diagnosis is transformation of uncomplicated malaria to a complicated one with multiple organ dysfunction and a very high mortality. We describe some of the clinical features, including the atypical ones, which may help in early diagnosis and prompt treatment. Children, pregnant women and non-immune adults are more susceptible to have severe and cerebral malaria. Several associated systemic complications like hypoglycaemia, hypovolemia, renal failure, disseminated intravascular coagulation, anaemia, lactic acidosis, and pulmonary oedema contribute to pathogenesis of coma and are responsible for high mortality. The clinical indicators of poor prognosis are young age, seizures, pulmonary oedema, and presence of organ dysfunction. Neurological sequel of severe malaria, and post-malarial neurological syndrome are important long-term problems in a significant minority even in adequately treated patients. Malaria in pregnancy poses significant problems of maternal, foetal and neonatal mortality. Pregnant women are more susceptible to develop complicated malaria. Recent reports allude to post-partum period also being one of the predisposing states to severe malaria. Lastly the recently highlighted explosive issue of interaction between HIV infection and malaria is described.

Malaria in Primitive Tribal population

Neeru Singh
Malaria Research Centre, Field Station,
Medical College Building, Jabalpur - 482003.

A longitudinal study was undertaken to define various epidemiological characteristics of malaria infection in five tribal forested villages of Mandla district (Madhya Pradesh). The objective was to determine the pattern of malarial infection and the annual and seasonal changes in the prevalence of infection with each *Plasmodium* species. A longer-term objective was to establish adequate baseline data for the evaluation of the effectiveness of various control measures, including future field trial of a malaria vaccine.

The study revealed that both *Plasmodium vivax* and *P. falciparum* were commonly prevalent in all the villages. Density of *Anopheles culicifacies*, an efficient vector was very high throughout the year. *An. fluviatilis*, another very efficient vector was also present in small numbers. Both the vectors were found breeding in slow running perennial streams and their tributories. Stream bed pools support very heavy breeding of *An. culicifacies* in all the study villages.

Analysis of the malariometric parameters investigated (1986-2000) revealed that though the prevalence of infection with each *Plasmodium* species showed variations from year to year, there had been a steady increase in the proportion of *P. falciparum i. e.,* from 25% in 1986 to 93.4% in 2000 (68% increase).

The factors potentially contributing to the upsurge in falciparum malaria were investigated. The evidence suggests that the advent and rapid spread of chloroquine resistance and decline in vector control measures might have contributed to the persisting high levels of *P. falciparum*. Further resettlement of population due to a multipurpose irrigation and hydroelectric project, difficult inaccessible terrain and a variety of socio-cultural factors may also be responsible for this upsurge.

S-3 ✓

Practices of Conventional Methods of Diagnosis, Treatment, Case Management and Control of Malaria Including Preparedness for Epidemic Control

Dr. Shiv Lal
Addl. Director-General of Health Services
Government of India

Dr. G. P. Dhillon

Malaria was a major scourge in India contributing 75 million cases with 0.8 million deaths in the pre-control era. The National Malaria Control Programme (NMCP) was launched in 1953 wich was extended to National Malaria Eradication Programme (NMEP) in 1958. By 1965 the programme brought down malaria incidence to 0.1 million cases and no deaths. Resurgence of malaria occurred with 6.4 million cases in 1976 necessitating introduction of Modified Plan of Operation (MPO). The Enhanced Malaria Control Programme (EMCP) was launched in Sept. 1997 with World Bank assistance covering high risk PHCs in Andhra Pradesh, Bihar (including Jharkhand), Gujarat, Madhya Pradesh (including Chhatisgarh), Orissa, Maharashtra and Rajasthan. In view of the change in the concept of the programme from eradication to containment, NMEP has been changed to National Anti Malaria Programme (NAMP).

The conventional methods of the diagnosis comprise of Active Case Detection (ACD) and activated Passive Case Detection (PCD) for Early Diagnosis and Prompt Treatment (EDPT). The Fever Treatment Depots (FTDs) and Drug Distribution Centres (DDCs) have been reactivated or newly established in endemic areas. Conventional methods of the treatment mainly comprise of presumptive treatment with chloroquine and radical treatment with primaquine while in drug resistant areas alternative drugs such as Sulfalene/Sulphadoxine + Pyremethamine are administered. Malaria case management of complicated *P. falciparum* infection has been highlighted giving the salient features of Revised Drug Policy.

Planning of interventional measures for transmission control requires selection of population for spray, timely procurement & supply of insecticides, change of insecticide due to vector resistance and operational aspects.

Prediction of malaria epidemics by monitoring regular data on environmental/climatic conditions, population movements in project areas/early detection of outbreaks and delineation of affected areas and prompt control measures have been enumerated to prevent epidemics.

Chemoprophylaxis of Malaria

Air Vice Marshal (Dr.) S. K. Dham, AVSM VSM, MBBS, MD (Med),
MSc Immunology (UK), FICA, FICP, FaeMS, Commandant
Command Hospital Air Force, Bangalore.

Malaria is one of the most common infections worldwide, especially in the tropical countries and the Indian subcontinent is an endemic area for the disease. There are four species of plasmodia that can cause human malaria, of which *P. falciparum* is capable of causing sever and fatal infections.

Chemoprophylaxis is a malaria control strategy adopted for limiting the morbidity and mortality associated with malaria in areas of high endemicity. This strategy is becoming increasingly difficult in recent years, owing to the emergence of drug resistant strains and the adverse effects of prophylactic drugs. Few areas of therapeutics are as controversial as antimalarial drug prophylaxis; experts disagree on whether chemoprophylaxis gives additional benefit if effective treatment facilities are readily available. However, it does have a useful role in reducing risk of infection in certain situations. Chemoprophylaxis is recommended at present in pregnant women, nonimmune travelers, and nonimmune persons living in closed communities in endemic areas e. g. armed forces personnel, policemen and labour forces.

The various classes of antimalarial drugs have been tried for prophylaxis but none can be expected to provide absolute protection. Thus chemoprophylaxis should be complimented with personal protection and vector control measures whenever feasible. The drug most widely used is chloroquine alone. It is used in combination with proguanil, dapsone and pyrimethamine in areas of chloroquine resistant Falciparum malaria. The emergence of multidrug resistant falciparum malaria has led to the use of mefloquine and doxycycline as chemoprophylactic drugs with both having comparable efficacy. The limiting factors are availability, cost and adverse effects of these newer drugs.

The recommendations of prophylaxis should be based on the knowledge of local patterns of drug sensitivity and risk of acquiring infection. Chemoprophylaxis is never entirely reliable and malaria should always be considered in the differential diagnosis of fever in patients who have traveled to endemic areas, even if they are taking prophylactic drugs.

S-5

Erythrocyte Invasion and Cytoadherence by Malaria Parasites

Chetan E. Chitnis
Malaria Research Group
International Centre for Genetic Engineering and Biotechnology
Aruna Asaf Ali Marg, New Delhi - 110067.

The invasion of erythrocytes by malaria parasites requires specific molecular interactions with host receptors. *Plasmodium vivax* uses the Duffy blood group antigen and *P. falciparum* commonly uses sialic acid residues on glycophorin A as receptors for erythrocyte invasion. The *P. vivax* Duffy binding protein (PvDBP) and *P. falciparum* sialic acid binding protein (also known as EBA-175) which bind these receptors during invasion share similar features and belong to a family of erythrocyte binding proteins. The extracellular regions of PvDBP and EBA-175 contain two conserved cysteine-rich regions, regions II and VI, which contain conserved cysteines and hydrophobic amino acid residues. The binding domains of PvDBP and EBA-175 lie in the N-terminal cysteine-rich region, region II. We are exploring the possibility of developing malaria vaccines based on these binding domains. Methods to express and purify the binding domains in their functional forms have been developed. Antibodies raised against *P. vivax* region II block the binding of PvDBP to human red cells and antibodies raised against the *P. falciparum* binding domain blocks erythrocyte invasion *in vitro*.

Following invasion, large molecular weight proteins referred to as PfEMP-1 are expressed on the surface of trophozoite and schizont-infected red cells. Expression of *var* genes, which encode PfEMP-1 undergoes antigenic variation. Some members of the PfEMP-1 family mediate the adhesion of *P. falciparum* infected erythrocytes to host endothelium. As a result, *P. falciparum* late stages sequester in the deep vasculature of host organs. Sequestration in brain capillaries is implicated in cerebral malaria. Binding to ICAM-1 is thought to be important for cytoadherence in brain capillaries. We have identified an expressed *var* gene from a *P. falciparum* field isolate that binds ICAM-1. The ICAM-1 binding domain was mapped to a cysteine-rich domain that shares homology with the *P. vivax* Duffy binding domain.

Malaria parasites thus use a conserved cysteine-rich domain to mediate erythrocyte invasion and cytoadherence, two processes that are central to malaria pathogenesis. The potential of developing malaria vaccines based on these functional domains will be discussed.

S-6

Properties of Vectors Affecting Malaria Transmission

Sarala K. Subbarao,
Malaria Research Centre
22, Sham Nath Marg, Delhi - 110054.

The genus Anopheles, one of the genera in Culicidae, occurs throughout temperate and tropical regions of the world. About 400 species are reported so far under this genus. Human Malaria parasites are exclusively transmitted by about 70 species of this genus.

In India there are about 58 anopheline species of which about nine species have been implicated in the transmission of malaria. *Anopheles culicifacies* transmits about 60-70% of all malaria cases annually. *An. dirus* and *An. minimus* are the important vectors in northeastern states, and *An. philippinensis* has a minor role in these areas. *An. stephrnsi* too has a wide distribution in India but attains importance in urban areas where it exclusively transmits malaria. *An. fluviatilis* is a very efficient vector of malaria in Western Ghats and Nilgris while it is not as efficient in east-central India, Assam and Himalayan foothills and is a poor vector in the south in Daccan plateu. *An. sundaicus* an important vector originally found in the coastal areas of Orrisa, West Bengal, Andhra Pradesh and Andamans is now restricted to Andaman and Nicobar Islands only. *An. annularis*, *An. varuna* and *An. jeyporiensis* are of secondary importance in malaria transmission.

Among anophelines in addition to commonly observed morphologically distinct species, there are species with a total reproductive isolation but morphologically indistinguishable. In our country, *An. culicifacies*, *An. fluviatilis*, *A. dirus*, *A. minimus*, *An. annularis* have been recognized as species complexes having isomorphic sibling species.

These species with their diverse characteristics in resting, feeding and breeding behaviour have occupied all varieties of ecological niches found in the country. Factors which influence the vectorial potential of mosquitoes namely feeding preference, longevity and man-mosquito contact due to their resting habits and biting rhythms of these species will be presented *vis-a-vis* the malaria situation in the areas of their prevalence.

S-7

The Use of Genetics in Vector Control

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Insecticide research led to the first, "Complete" – victories in combating pest almost 50 years ago with chlorinated hydrocarbons followed by the organophosphates, methyl carbamates and pyrethroids – all neuro active chemicals. The very wide spread use of insecticides for pest control is largely a result of their convenience, simplicity, effectiveness, flexibility and economy. However, the insect vectors have developed resistance for the insecticide and their continuous use contributed to environmental pollution. Therefore, it is imperative that alternative control measure may be developed which does not involve resistance. Genetic control is one such method.

One of the basic requirements of vector control through genetic manipulation is to establish basic genetic information especially those traits/genes responsible for insecticide susceptibility, disease transmission, recognizing the host at right time *etc.* Such studies could be achieved through studies on cytology, cytogenetics including establishment of mutant markers *etc.*

Chromosomal translocations and pericentric inversions are considered potential means of controlling insect vectors. Genetic sexing strain involving male linked translocation, conditional lethal and incorporation of crossover suppressors can be utilised to produce only males and thereby it can be used in the Sterile Insect Technique (**SIT**) programme of insect vectors of diseases. Beneficial genes such as genes for refractoriness can be used in the production of transgenic strain and thereby used in the control of insect vector of diseases.

Molecular Biology of the Malarial Vector Mosquito

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Mosquitoes of the genus *Anopheles* transmit malaria to humans. This vectoral specificity for parasite propagation could be understood if one identifies the molecule(s) encoded by the host mosquito in response to parasite load. In order to probe into the mosquito genome, molecular and genetic tools capable of manipulating the genome are necessary. Only recently, *i. e.* in the last year, successful germline integration of foreign DNA into *An. stephensi* could be achieved using a *Drosophila* retroposon element, Minos, derived DNA sequence. In our laboratory, over past few years, we have isolated & characterized a few middle repetitive genomic sequences related to transposons from *An. stephensi*. These sequences were used for integration of exogenous DNA mediated by homologous recombination when incorporated in a recombinant plasmid and using mosquito cell culture. Such a construct is potentially a desirable candidate for germ line transformation. We have also succeeded in introducing and expressing reporter gene based constructs with GFP or β - galactosidase by injecting them in the fourth instar larvae. Their expression was detected in larval and adult tissues. Additionally, we have begun isolation and localization of a few more genes such as newly identified *Drosophila* hexokinase and aminopeptidase genes on *Anopheles* chromosomes. Yet another novel gene coding for a 122 aa chain was localized on chromosome 2 of *An. stephensi*. This gene codes for a DNA binding protein having helix turn helix motif similar to homeodomain binding proteins. Its function in *Drosophila* appears to be in early differentiation of primpodial cells of PNS.

Parasite induced mosquito genes are the key to trace compatibility and refractivity phenotypes. Since, Schneider & Shahabudeen have recently shown that *Plasmodium gallinaceum* could be developed in *D. hydei*, it will be more convenient to identify the corresponding *Drosophila* genes and their functions so that they could be related in mosquitoes. We believe that such genes will not be unique genes, but will be re-recruiting of the existing gene functions towards parasite compatibility in permissive vectors. This hypothesis is based on our observations related to adaptive cross-tolerance to different stresses and hsp's. Multifunctionality of genes is here to stay.

Drug Resistant Malaria

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Malaria is a threat to almost half the world's population. Resistance to commonly used drugs has been a serious problem and hindrance to control of morbidity, mortality and incidence. Multidrug resistant strains of *P. falciparum* blood stages have been reported from many parts of the world. In India, resistance to chloroquine, sulfadoxine-pyrimethamine, quinine, mefloquine, and the artemesinin derivatives have been reported. *P. vivax* though not a killer malaria, nevertheless causes considerable morbidity. This is due to its relapsing character it is also the commonest form of malaria seen in India. *P. vivax* resistant to chloroquine is approximately 1 – 2% and has been reported from Mumbai and other areas in India. Use of primaquine to prevent relapses has been an important control measure. However over the past 20 years efficacy of primaquine has decreased substantially. In 1977, an adult dose of 15 mg per day for 5 days led to relapse in 1% patients. However in 1997, relapse occurred in 13% patient with same dose, in 0% with 15 mg per day for 14 days. Now in year 2000 – 2001 relapse with 15 mg primaquine per day for 14 days is already 5% (relapse with no primaquine is 11.7%)

Use of gametocytocidal drugs is a commonly advocated measure to prevent transmission. However efficacy of primaquine at 45 mg dose (currently the only effective Gametocytocidal drug for *P. falciparum*) has now decreased with 26% of patients with chloroquine sensitive parasites and 65% with chloroquine resistant (RI) parasite showing gametocytemia at day 29 after chloroquine primaquine treatment. *In vitro*, gametocyte exflagellation technique demonstrated viability hence capability of transmission. Newer drugs and combination are needed for treatment of clinical attack, prevention of relapse and gametocytemia. Co-artemether, bulaquine, (to be marketed and marketed respectively) have been investigated by our team. Continued surveillance for resistance in both species is needed.

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Experimental Malaria Models: Application to Drug Research

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Malaria still remains one of the most prevalent endemic parasitic diseases of the world. Clinical and experimental evidence in recent years have amply demonstrated the parasite's ability to develop resistance to a variety of antimalarial drugs. There is a continued need for improved drugs for the management of resistant parasites, and in addition, novel drugs with causal prophylactic activity are especially needed for prevention of malaria. The need for new chemotherapeutic agents over the years has resulted in use of avian, rodent and simian parasites as experimental models, since, unlike the other major tropical diseases, the causative organism of human malaria can not be maintained in small laboratory animals. Until the discovery of murine parasites in 1948, the avian models served a distinctly important role to assay the efficacy of potential agents. However these screens were of limited value due to the differences exhibited by the avian parasites in the choice of their exoerythrocytic host cells and in exhibiting a repetitive exoerythrocytic phase, which also gets initiated from the erythrocytic merozoites. Rodent models have played a major part during the last 50 years in enhaning our knowledge in malaria chemotherapy including aspects on drug resistance. Several simian malaria parasites, due to their advantages in closely simulating the biological pattern of infection in human host, have served as excellent models for chemotherapeutic studies.

Following the resurgence of malaria in India during 1970-1975, Central Drug Research Institute initiated new drug development program with an aim to develop new drugs to combat drug resistant parasites, safe primaquine substitutes for control of relapses in *P. vivax* and new gametocytocidal agents to stop transmission. A high priority was accorded to establish reproducible assay protocols in rodent and simian models for systematic pre-clinical development. This presentation aims to review the relevance and application of these models in rational drug development.

Future Therapeutic Targets to Treat Malaria

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The antimalarials available today can be put into two broad categories. One group is based on natural plant sources such as Cinchona and *Artemisia annua* giving rise to founder molecules such as quinine and artemisinin respectively. A whole battery of compounds belonging to 4 - aminoquinolines, 8-aminoquinolines, trioxanes and tetraoxanes have been synthesized based on the structures of the founder compounds. The second class of compounds are those designed against classical targets such as folate pathway, purine (salvage) and pyrimidine pathways. The main problem is one of development of resistance to both the classes of compounds. However, it is difficult to relegate any particular pathway or target as no more useful, since newer knowledge may present it in a different light to embark on fresh approaches to design new molecules. Chloroquine and aminoquinoline analogues are good examples in this regard. Nevertheless, there are new and emerging targets based on unique features of parasite biology such as food vacuole and hemoglobin catabolism, *de novo* heme biosynthesis and protein import, apicoplasts and fatty acid biosynthesis, gene expression in apicoplasts, membrane potential in mitochondria, cytoadherence and potential inhibitors of the process. Thus, apart from the aminoquinolines, sesquinterpenoids and folate analogues a new generation of compounds such as atavaquone (mitochondrial membrane potential), glyphosate (shikimate pathway), triclosan and thiolactomycin (fatty acid biosynthesis), peptides (specific protein transport and heme biosynthesis), clindamycin, thiostrepton, amythamicin etc. (plastid translation), chalcone and phenothiazine (protease inhibitors) fasidomycin (isoprenoid biosynthesis) etc. are available as candidate antimalarials. Further research only will tell whether we will have a cheaper alternative to chloroquine.

S-12 ✓

Genomics and Drug Development in Malaria

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Antimalarial drugs saved millions of lives in the twentieth century. Now, owing to the evolution of drug resistance, there is an urgent need for a new generation of antimalarials. The major emphasis is on the identification of novel metabolic pathways of the parasite.

Knowledge of the sequence of the *Plasmodium falciparum* genome and genomes of other parasites that cause malaria, as well as the specifics of gene and protein expression at different stages in the life cycle and under pressure from different drugs has increased our chances of developing new and better drugs and vaccines.

Example of use of functional genomics in identifying a unique target and inhibitor in *P. falciparum* would be discussed.

S-13

Novel Target Proteins of *P. falciparum*

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It is documented that people living in malaria endemic areas acquire immunity against malaria after repeated infections. Studies involving passive transfer of IgG from immune adults to the non-immune subjects have shown that circulating antibodies play an important role. Through a differential immunoscreen, we have identified several novel cDNA clones, which react exclusively and yet extensively with immune sera samples. Specific antisera and other combinatorial reagents raised against the immunoclones, inhibit the growth of parasites in culture. The clones studies so far, turn out to be novel conserved *Plasmodium* genes, and each of the proteins encoded is localized on the parasite surface. Therefore these are possible targets of invasion blocking antibodies. The properties of three of the clones, Pf4 (PfP0), Pf9 and Pf2, will be discussed. Peptides based on Pf2 and Pf9 have been used for the study of these proteins. Lymphoproliferative T-cells, to the Pf2, Pf9 and PfP0 peptides, were observed during the course of infection in mice with the murine malarial parasites, *P. berghei* and *P. yoelii*. Molecular epidemiological assays of the response to some of these peptides have been performed on the immune adults of South Kenya, and these show as much immune response as MSP1 and EBA peptide domains. This demonstrates a natural immune response to these conserved epitopes.

S-14

Immune Mechanisms in Malaria: Role of Vaccine in Control

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Malaria immunity, when develops in the host in response to infection with Plasmodium, is highly complex. Not only these responses are specific to various stages of development of the parasite, but are also characterised by several immune invasion mechanisms developed by the parasite. A notable fact is that acquired malaria immunity is short-lived, and is characterised by very complex interplay of various cytokines. It is now clear that cellular immune responses play a crucial role in malaria immunity, and these mechanisms will have to be clearly understood before establishing rational basis for malaria vaccine development. A relatively large number of malaria antigens for vaccine development have been characterised and it is not yet clear if any one of them will be a clear vaccine candidate. We have been involved in characterising the immune responses to specific malaria vaccine candidates with respect to human malaria *Plasmodium falciparum*, and for monkey simian malaria *P. cynomolgi*. Procedure to produce three different antigens from the blood stage of the parasite by recombinant methods have been developed. Simultaneously, suitable DNA delivery vectors containing the genes for some of these antigens have been made, and a comparison of the two strategies of immunisation i.e. protein and DNA immunisation, have been carried out. Some of these results and the above issues will be discussed in details.

Beyond Biomedicine: The Challenges of Socio-Epidemiological Research

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The resurgence of malaria in the last two decades has become a matter of serious concern for health professionals, health researchers, policy makers and planners. The growing evidence of epidemics from many parts of the country; the increasing spread of *Plasmodium falciparum* infections around the country; and the changing patterns of resistance to antimalarials and insecticides have been recognized as new challenges.

The classical bio-medical approach has dominated public health and medical research in this country with the focus on resistance to the existing tools for malaria control and the development of new tools including vaccines. Research in these directions has been encouraged by the changing frontiers of molecular biology and genetics. Even WHO and other international agencies supporting Malaria research have focussed on "a) Specific tools to reduce malaria transmission and prevent or cure malaria, and b) Operational research evalidation of tools in field conditions". The quest for 'magic bullets' goes on.

However, there is growing evidence that malaria resurgence is linked to broader social trends not only biomedical imperatives. Firstly the resurgence is being recognized as symbolic of a ***collapse of the 'Public Health system'*** as well as a gross ***distortion of the primary health care - strategy*** which has taken place all over the world. While factors such as the preoccupation with secondary and tertiary health care at the cost of primary; inadequate epidemiological orientation of the health team and poor logistical planning are known factors that contribute to this, there are newer agents as well. Recent liberal economic policies have led to a ***decreasing investment in health*** and a rolling back of state support and intervention in public health leading to shortages of human power and inadequate surveillance.

Secondly the continuing ***irrational management of suspected malaria cases*** by general practitioners and specialists by a plethora of irrational injections, antipyretics and even antibiotics, and this inspite of Rational care guidelines by NAMP and WHO has contributed to increasing cost of care and complications as well as perhaps to the development of resistance.

Thirdly international public health strategies more recently have been ***promoting single socially marketed magic bullets*** like insecticide treated mosquitoes nets (ITMNs) neglecting more comphrensive and plural approaches and strategies including bio environmental control and indigenous or traditional approaches.

An independent expert group of which the author was the chairperson made an assessment of the malaria problem taking a broader social – economic –cultural political

approach to epidemiological analysis. Apart from the factors, mentioned above it identified a host of others contributing to the resurgence. These included malariogenic ***development strategies; migrant labour; agricultural development without health impact assessment; increasing loss of public health skill and competence in the health care system; inadequate involvement of private health practitioners, voluntary agencies and the community in the programme; increasing corruption and political interference in the choice of tools and strategies and so on.*** What is urgently required therefore are action research studies; KAP surveys and socio-epidemiological surveys; studies on the political economy of malaria control to better understand the problem.

This papers builds on the above assessment and makes a case for greater focus on studying the community at risk including social – cultural - economic – political and behavioral issues as a complementary strategy to the ongoing biomedical research focussed on agent and vector.

In the absence of this shift of emphasis, malaria and reemerging communicable diseases will continue to represent not only a failure of our public health system but also a failure of our research methods. Socio - epidemiological and social /economic policy research is the need of the hour.

Control of Malaria Transmission Using Larvivorous Fishes

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Demonstration of malaria control using larvivorous fishes was carried out in a silk producing area of PHC Kamasamudram (93 villages, pop. 36,627) district Kolar, Karnataka. Geographical Reconnaissance of larval breeding habitats revealed that irrigation tanks, wells and streams are the major breeding habitats for the vector mosquitoes *An. culicifacies* and *An. fluviatilis.* Percent contributions of these species from irrigation tanks were 8.8 and 5.4, from wells 42.5 and 53.9, from streams 42.3 and 38.5 respectively. Intervention with larvivorous fishes Guppy (*Poecilia reticulata*) in wells and streams in 1994 and *Gambusia affinis* in 1996 in tanks were found suitable. These fishes got well established in these ecosystems. Entomological monitoring revealed that significant impact on the larval densities of all mosquitoes was observed ($P<0.001$). Larval emergence data revealed that significant impact was observed on the vector species in all the breeding habitats ($P<0.001$) while no impact was recorded on *An. fluviatilis* from streams ($P<0.05$). There was significant impact on MHD of *An. culcifacies* while no impact was observed on *An. fluviatilis*. Sibling species studies of the two vector species revealed that species A of *An. culicifacies* was prevalent in the villages having wells (~90%) while species B in stream influenced villages (~90%). All the *An. fluviatilis* were found to be species T. Blood meal analysis indicated that *An. fluviatilis* was zoophagic while *An. culicifacies* marginally anthropophagic (HBI, 0.08). Impact on malaria incidence was highly significant. API was 41.8 in 1993, 15 in 1994, 11.1 in 1995, 12.3 in 1996, 4 in 1997, 1.2 in 1998, 0.1 in 1999 and 0.005 in 2000. This method was found most economical than any other methods.

Vaccine Research in India

March 11, 2001

Edward Jenner (1749-1823), after training in London and a period as an army surgeon, spent his whole career as a country doctor in his native county of Gloucestershire in the West of England. His research was based on careful case-studies and clinical observation more than a hundred years before scientists could explain the viruses themselves. So successful did his innovation prove that by 1840 the British government had banned alternative preventive treatments against smallpox. "Vaccination," the word Jenner invented for his treatment (from the Latin *vacca*, a cow), was adopted by Pasteur for immunization against any disease.

In the eighteenth century, before Jenner, smallpox was a killer disease, as widespread as cancer or heart disease in the twentieth century but with the difference that the majority of its victims were infants and young children. In 1980, as a result of Jenner's discovery, the World Health Assembly officially declared "the world and its peoples" free from endemic smallpox.

(*Source: URL: http://www.sc.edu/library/spcoll/nathist/jenner.html Copyright © 1999, the University of South Carolina*)

Abstracts

March 11, 2001

The Development and Value of Cholera Vaccines

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Vibrio cholerae is the causative agent of the disease cholera. Despite continued efforts to provide clean drinking water and basic sanitation, the number of cases and number of countries reporting cholera to the World Health Organisation has shown a precipitous increase in the 1990s. The emergence of cholera as a major public health problem throughout the world has prompted a renewed effort to develop a safe and effective cholera vaccine. It should be emphasized that a cholera vaccine is considered only as an additional tool to prevent cholera and will not replace any of the other cholera prevention and control interventions such as treated water supplies, improving water delivery and sewage control, hand washing facilities, latrines and adequate hygiene in food handling.

Since the withdrawal of the traditional injectable cholera vaccine in 1973, substantial progress has been made in the development of oral cholera vaccines. Two of these new oral cholera vaccines have already been on the market for some years. One of the recently developed oral cholera vaccine is the killed whole cell B subunit vaccine which was developed in Sweden and licensed in 8 countries while the other vaccine is the live attenuated oral cholera vaccine produced in Switzerland and licensed in 9 countries. Among the new-generation cholera vaccines, convincing protection against cholera in field situation has been demonstrated only for the oral B subunit killed whole-cell cholera vaccine. These two recently developed oral cholera vaccines are, however, too, expensive for general public health use in developing countries and so far only used and afforded by travellers and tourists.

In view of the high cost of currently available cholera vaccines, a concerted multi-laboratory collaboration was initiated in India in 1995 to develop an indigenous live oral cholera vaccine. This collaboration has resulted in the construction of a new live oral candidate vaccine from a non-toxigenic clinical strain of *Vibrio cholerae* E1 Tor, Inaba devoid of the CTX prophage but which posses the *tcpA* (toxin coregulated pilus which is an adhesin) and *toxR* (a regulatory transmembrane protein) genes. Through a series of genetic manipulations, the *ctxB* (B subunit of cholera toxin) gene of *V. cholerae* was introduced into the cryptic hemolysin locus of the strain. By Beads-ELISA, the resulting strain named as VA (Vaccine Attempt) 1.3, was found to produce copious amount of the B subunit of cholera toxin but did not cause any fluid accumulation in the rabbit ileal loop assay. VA1.3 was non-reactogenic and provided complete protection against challenge doses of both classical and E1 Tor biotypes of *V. cholerae* O 1 when examined by the RITARD model and also elicited significant anti-bacterial and anti-CTB antibodies. Phase 1 and 2 trials are ongoing with this recombinant oral cholera vaccine and initial results have shown VA 1.3 to be safe and non-reactogenic vaccine.

Vaccine Strategies Against Rotavirus Diarrhoea

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Rotavirus is the major causative agent of acute infantile dehydrating gastroenteritis in humans and a variety of animal species. Rotavirus disease accounts for about 800,000 deaths annually, of which India accounts for about a fifth of this tragedy. Rotavirus also causes severe economic loss to the farm and dairy industry. In spite of intense international efforts, no effective vaccine is yet available. Several strategies are being explored to control of the rotavirus disease. Of these, live attenuated/asymptomatic reassortant vaccines are being actively developed and are at various stages of clinical evaluation in different countries. We have isolated an asymptomatic strain I321 from normal newborn children in Bangalore. I321 is an unusual strain in that it has evolved by gene reassortment between a G 10P11 type bovine rotavirus and a human virus in nature. Long-term epidemiological data suggests that about 60 percent of newborn children get infected by I321 in hospitals in Bangalore. This high rate of asymptomatic infection of neonates appears to have resulted in a significant reduction in rotavirus diarrhoea. I321 has been proposed as a potential candidate for live vaccine in humans and phase I clinical trials are being initiated.

Although subunit vaccines based on capsid proteins were pursued in 1980s, this approach has been now abandoned as the neutralization epitopes on the major outer capsid protein VP7 is highly conformation-dependent and requires co-expression of other capsid proteins to form the natural epitopes. In this context, attempts are being directed to produce virus-like particles by co-expression of the capsid proteins in baculovirus expression system for use as vaccines. But this approach will be highly expensive.

The other approach that is actively being examined is to target the nonstructural proteins that are critical virus replication, translation and morphogenisis. One of the candidates is the multi-functional protein NSP4 which has been implicated as rotavirus enterotoxin. This could serve as a potential candidate for subunit vaccine as well as a target for structure-based drug design. The current status of these various approaches will be discussed.

Epidemiology of Rotavirus Infection in India

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Rotavirus is the leading cause of childhood diarrheal diarrhea worldwide, causing an estimated 600,00 deaths each year. To assess the potential benefits of a national rotavirus immunization program in India, we analyzed 40 published studies of rotavirus that were conducted between 1976 and 1997 and included a total of approximately 13,000 Indian pediatric inpatients. Pediatric studies featuring 100 or more patients and lasting for at least 12 months in duration and all neonatal studies were analyzed. Rotavirus was detected in a median of 18% of pediatric patients and 28% of neonates surveyed. Fifty percent of all children hospitalized with rotavirus by age 5 were hospitalized by the age of 6 months, 75% by the age of 9 months, and almost 100% by the age of 2 years. Rotavirus was most prevalent (31%) in children between 7 and 12 months of age, followed by children between 1 and 2 years of age (20%), and children <7 months of age (13%). The P[11] type is common among animal rotavirus in Delhi and the unique neonatal strains probably arising by reassortment. VP7 genotypes G1 and G2 were most commonly isolated although significant heterogeneity of serotypes was observed. P[11], G9 strains were most frequently isolated among neonates but without diarrheal symptoms. P[6], G 9 strains were common in the only multicentre evaluation of rotavirus serotypes in hospitalized children with acute diarrhea in India. In 1993, 15 of 63 and in 1994-1995, 21 of 93 rotavirus strains were of G9 type. P[11], G9 isolated in neonates were rarely found outside the neonatal period and that to only in hospital settings. G9 strains from neighboring countries India and Bangladesh were less homologous at the level of nt and aa sequences (VP7 gene) and had independent lineages by phylogenetic analysis indicating that the much greater rotavirus diversity among rotavirus population is generated by reassortment between gene segments than by accumulation of spontaneous mutations.

Based on India's 1998 birth cohort of 24,671,000 infants and 1998 under 5-mortality rate of 105 per 1000, a total of 2,590,000 deaths in children under 5 are estimated to have occurred in 1998. Of these 544,000 are associated with diarrhea. Based on a median 18% of children hospitalized with diarrhea, and assuming a proportionate contribution to diarrhea deaths, we expect 98,000 deaths due to rotavirus in India annually.

These data underscore the urgent need for safe and effective interventions against rotavirus such as vaccines. The significant diversity of rotavirus strains and

young age of hospitalization poses unique challenges to the formulation of a rotavirus immunization programme in India. Neonatal strains have been shown to be immunogenic and protective in natural infection studies and that may be potential vaccine candidates.

Prospects of an Indian HIV-1 subtype C DNA Vaccine

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Molecular epidemilogy of HIV strains circulating in India has indicated that HIV-1 subtype C is the predominant virus clade. Further analysis suggested that majority of subtype C viruses was of the South African variety (C3). Zambian variety (C2) was also found in our study. Therefore, it is imperative that a vaccine based on the local circulating subtype should be designed. We have cloned DNA segment encoding HIV-1 gp120 genes from two Indian isolates of HIV-1 subtype C into mamalain expression vector, pJW4304. These constructs (pJWCh18 with subtype C3 gp120 and pJWSK3 with subtype C2 gp120 gene segments) were used for immunization of mice. Humoral as well as cell mediated immune responses were measured in immunized animals. Effector cells from mice immunized intramuscularly with these constructs showed MHC class I restricted cytotoxic T lymphocyte (CTL) activity against homologous as well as heterologous peptide (from env V3 region) pulsed target cells (P815, H2 compatible mouse mastocytoma cells) thus demonstrating cross clade reactivity. *In vitro* lymphoproliferative response and Th1 cytokine response to HIV antigen stimulation of spleen cells from immunized mice were seen with high levels of IFN-γ and IL-2 but undetectable IL-4 and IL-5 production. However, immunized mice produced low levels of antibodies against gp120.

In addition, we have also cloned gag-protease gene fragment from an Indian HIV-1 subtype C strain into the expression vector, pJW 4304. This plasmid (pJWgag-protease) produced p24 antigen in Cos7 cells upon transfection. Presently, it is under evaluation for immunogenicity in mouse model. Preliminary data suggest that this construct also induces cell-mediated immunity in mice even after two doses. Currently, experiments are also underway to clone gp120 as well as gag protease gene in MVA vector. We are also in the process of evaluation of these HIV-1 *env* DNA constructs the immunogenicity and protective efficacy in non-human primates at Primate Research Centres at Bangalore and University of California at Davis, USA, respectively.

Recombinant Vaccines for Japanese Encephalitis

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Japanese encephalitis, commonly known as brain fever, is caused by Japanese encephalitis virus (JEV) which belongs to family *flaviviridae* of animal viruses. The virus is transmitted to human beings by mosquitoes. A mouse brain-grown, formaline-inactivated JEV vaccine is presently available internationally. However, this vaccine has limitations in terms of its high cost, safety and efficacy. Attempts are, therefore, being made to develop recombinant DNA based vaccines with improved safety and efficacy.

JEV envelope protein has been shown to contain the virus neutralization antigen and antibodies to it were shown to provide protection against JEV infection in experimental animals. Recombinant envelope protein of JEV, synthesized in mammalian cells, *E. coli* or baculovirus, induced JEV neutralizing antibodies in mice but provided only low levels of protection against lethal JEV challenge. Recombinant pox viruses expressing JEV proteins induced high titers of JEV neutralizing antibodies in mice and provided high levels of protection to these animals against lethal JEV challenge. However, these recombinant viruses failed to generate significant JEV neutralizing activity in human volunteers. Recently, a chimeric virus expressing JEV envelope and pre-Membrane protein on the background of the Yellow Fever 17D vaccine virus has been described. The chimeric virus shows great promise as a potential recombinant JEV vaccine as it provided significant protection to immunized mice and rhesus monkeys against lethal JEV challenge. In last couple of years a number of plasmid DNA constructs expressing JEV proteins have been described that provided a range of protection to immunized mice against lethal JEV challenge. These DNA vaccines, while promising, require improvements in terms of their immunogenicity and efficacy. This lecture will review efforts by various groups to develop recombinant JEV vaccines and discuss our work on the development of plasmid DNA based vaccine against JEV.

Protection Studies in Rhesus Monkeys Against Hepatitis E Virus (HEV) by Recombinant Protein Immunization

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Hepatitis E virus (HEV) is the major human pathogen in the developing world responsible for an acute and self-limiting viral hepatitis. HEV is a non-enveloped 27-32-nm diameter positive stranded RNA virus of 7.2-kb genome consisting of 3 ORFs.

The study was carried out to evaluate the protective immune response in experimental animals (rhesus monkeys) following immunisation with HEV recombinant proteins (i. e., non structural protein pORF1 consisting of protease, RNA dependent RNA polymerase (RdRp) and structural protein pORF2, pORF3).

The rhesus monkeys were immunized with a mixture of both structural and non-structural proteins and were challenged with HEV for possible protection. Antibodies against different viral proteins were detected qualitatively and quantitatively by using in-house enzyme immunoassay.

The Rhesus monkeys (12) were immunized with 100 micrograms of each of the above mentioned recombinant proteins with alum at 0, 29, 48 and 84 days. The monkeys developed IgG antibody titre of 6000-8000 after 3rd booster. The animals challenged at 94th day showed no protection against HEV infection as evidenced by detection of viral RNA in serum, bile, liver and fecal samples. The IgG antibody titre declined to low level between 4-6 months. The remaining animals challenged at the end of 8 months again showed no protection. The T cell proliferative response against protease and RdRp was elicited in 2 animals.

The monkeys immunized with the recombinant proteins although developed good titre of antibody showed no protection. The titre of IgG falls significantly between 4-6 months. There was no significant T cell response seen.

DNA Vaccines

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A novel approach to the development of needed vaccines uses DNA for immunization. DNA represents the genetic blueprint for life. When DNA is used for immunization, the DNA in plasmid form provides the code for the vaccinating protein. The actual production of the immunizing protein takes place in the DNA-inoculated host, initiating both humoral and cellular immunity. DNA vaccines are administered in saline using hypodermic needles or by propelling DNA-coated gold beads into skin using gene guns. Recent results obtained in animal models indicate that this new technology may revolutionize the vaccination of humans. Protective immunity has been achieved for such major killers as diarrhea-causing viruses, tuberculosis-inducing bacteria, and malaria-inducing parasites. These new DNA vaccines also hold promise for being safer, less expensive, and easier to produce and administer than conventional vaccines.

Establishing a Commercial Vaccine Production Facility- The Serum Institute's Experience

Dr. S. V. Kapre
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A labscale process involving vaccine development in itself is exciting to develop. But it presents a bigger challenge when one has to scale it up to industrial scale.

Issues of process equipment, time schedule, downstream processing, and environment control etc. come in force. When the product is parenteral, this becomes extremely complex, as the limits of control are extremely stringent. Then to design a manufacturing station becomes challenging, as one has to grapple with mundane nitty-gritty with the same alertness, as the designing of the process needs.

Grappling with market forces is another challenge and a mechanism to meet the demands is another feat that needs to be achieved.

Thus stretching human skills beyond the science of biotechnology becomes inevitable which makes this very exciting and gives a sense of complete satisfaction.

The Need for an Alternative to BCG

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Despite the discovery of the tubercle bacillus more than a century ago, and all the advances made since then, tuberculosis still remains as the leading cause of death, particularly in developing countries. About one third of the world's population is infected with *M. tuberculosis*. It is estimated that at present there are about 9 million new cases of tuberculosis with 3 million deaths. India accounts for nearly 30% of all TB cases in the world and more adults in India die from TB than from any other infectious disease. The only existing vaccines against tuberculosis is the BCG, and as of now it is mandatory or officially recommended in more than 180 countries or territories across the globe. More than 3 billion doses have been administered over five decades with minimum side effects. Although it has been proved as a stable, immunogenic, inexpensive and a safe vaccine, its efficacy is highly variable, ranging from 0-80%. Outcome of two major community based trials carried out in Madanapalle and Chingleput district in South India showed that BCG had no effect in lowering the total number of infectious adult forms of bacillary pulmonary tuberculosis in the community. However, recent retrospective studies of BCG Vaccine efficacy among newborns and children have reported a protective effect against all forms of tuberculosis ranging from 17 to 90%, and protection against tuberculosis meningitis and against cavitary, military and bone and joint tuberculosis has been estimated to be about 75% or greater.

Based on voluminous data accumulated so far on BCG vaccine, it is clear that the present vaccine, cannot substantially influence the epidemiological situation but should be continued for children since there is evidence of its interference with the haematogenous spread of tubercle bacilli, thus reducing the risk of severe extrapulmonary manifestation.

To overcome the limitations of existing vaccine, fewer strategies have been envisaged. These include construction of live attenuated mycobacterial vaccines which would mimic natural infection without evoking pathological sequelae; manipulation and improvement of BCG vaccine itself to express major secretary proteins to induce adequate protective immunity against tuberculosis; development of subunit vaccines to boost the immune response against important antigenic determinants responsible for protective immunity or usage of DNA vaccines encoding the relevant antigens necessary for protection against disease. Since the entire genome sequence of

M.tuberculosis has been known, effortrs are underway by many researchers to address the vaccine issues by employing the above mentioned strategies. The available evidence on the current developments will be presented.

Immunology of Tuberculosis: Possible Approaches for a Vaccine

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Mycobacterium tuberculosis carries out a constant dialogue with its infected host where disease results when the organism has the last word. The ability of the plethora of unique proteins, cell wall lipids, carbohydrates, glycoproteins, glycolipids, lipoproteins etc. of this successful pathogen to stimulate the multiple arms of the immune system is legendary, as evidenced by its use in the formulation of complete Freund's adjuvant. It is ironic that this organism, when live, is primarily an immune suppressing agent, as evident in TB patients. Nevertheless, the tubercle bacillus elicits a potent primary T cell response even in those individuals who subsequently succumb to the infection. For reasons still not fully understood, this initial response characterized by the beneficial TH1 profile takes a turn in the direction of the ineffective TH2 profile in those unfortune individuals who fail to contain the infection. The immunology of tuberculosis has therefore been pursued vigorously with the hope that the insights gained would help to prop the immune system to successfully combat the initial infection as well as prevent reactivation of latent infection.

The early studies of George Mackaness showed unequivocally that lymphoid cells, but not B cells mediate protection against TB. Adoptive transfer studies carried out in mice made resistant to TB by a low dose infection with the tubercle bacilli revealed the protective role of both CD4+ as well as CD8+ T cells in containing the TB bacilli. It was however not clear how a bacterium that enters the macrophage primarily through phagocytosis or endocytosis could possibly elicit any CD8+ T cells which was known to require antigen synthesized endogenously within the host cell. The availability of mouse strains disrupted in the gene for β2-microglobulin helped to prove that CD8+ T cells that are stimulated by antigen presented on self MHC class I molecules are essential for developing resistance to TB since these mice died very early following challenge with virulent *M. tuberculosis* and could not be protected by BCG. Subsequently, elegant studies from the Bloom laboratory showed that co-infection with live *M. tuberculosis*, but not dead bacilli allows presentation of exogenously added soluble proteins on MHC class I to CD8+ T cells, proving that *M. tuberculosis* infection of cells allows soluble proteins to access the class I MHC presentation pathway. The importance of CD8+ T cells in protection against TB was confirmed when it was shown

that mice with a disruption of the peptide transporter (TAP) gene also succumbed early to *M. tuberculosis* infection.

It is now becoming clear that the mycobacterial phagosome within which the bacteria reside while growing inside the macrophage, does allow exit of several proteins into the cytosol, either inadvertently or intentionally to mediate the down regulation of host cell functions. An important role for the mycobacterial hemolysin in this leakiness of the phagosome membrane has been suggested. It is tempting to speculate that secreted proteins of *M. tuberculosis*, would be used by the organism to mediate its virulence functions by leaving the phagosome, and in so doing, gain access to the MHC class I antigen presentaton pathway, thus, stimulating cytolytic T cells. The secreted proteins would thus function like the proverbial Achilles' heel, mediating pathogenesis for the bacterium while simultaneously mediating its vulnerability to host immune responses.

We have therefore focused on identifying proteins of *M. tuberculosis* capable of eliciting CD8+ T cells in the exposed endemic population. Interestingly, these have predominantly turned out to be secreted proteins. It would follow from the above argument that immunization with these antigens in a manner that can elicit CD8+ T cells should protect against a challenge with virulent *M.tuberculosis*. This surmise appears to be holding true in our studies, and might give rise to the delineation of perforin-producing CD8+ T cells as a reasonable correlate of protective immunity to TB. Our current studies will also attempt to demonstrate direct killing of *M. tuberculosis*-infected target cells by these antigen specific CD8+ T cells.

Field Trial of Leprosy Vaccines

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Elimination of leprosy is almost achieved at the global level by the end of the year 2000 and is expected to be achieved at the national level by 2005. It is difficult to perceive that hardly 2 decades before, with the looming danger of dapsone resistance, the first priority for leprosy research was development of an effective prophylactic vaccine. Today, multi-drug therapy against leprosy is a success story after the only success of penicillin against yaws, using chemotherapy, to control, eliminate or eradicate a communicable disease. Against this background it is hoped that the successful, meticulously conducted South India Leprosy Vaccine Trial would be useful in the field of vaccinology and for the final onslaught towards eradication of leprosy.

BCG has been tried as a leprosy prophylactic vaccine since 1940s. There were 4 major trials using BCG in the 60s giving varied results. In the context of India BCG had very limited success of about 25% against leprosy of all forms, but no impact against the smear positive forms. In the 80s there were a series of newer vaccines against leprosy, viz. BCG + armadillo derived *M. leprae*, ICRC and Mw. At least 5 field studies were conducted to assess their efficacy. South India Vaccine Trial was one where all these three candidates were field tested for their prophylactic efficacy. This trial was preceded by a series of pre-clinical studies in animals, both to prejudge efficacy and also to test for toxic effects. After these candidates were cleared for human use, clinical trials have been conducted in phases, finally leading to the Phase III study in Kancheepuram and Tiruvallur Taluks in Chingleput district in Tamil Nadu. This large field study covering 300,000 people, over a period of almost a decade provides a huge opportunity to examine several issues regarding ethics, trial design, conduct, controversies and relevance. In my presentation, I will dwell on these issues from the point of view of possible future vaccine prophylaxis studies in more general sense and not restricting to leprosy alone

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(10) Dr Deshmukh

Vector — Specificity | Refractivity parameters
— Resistance

(11) Dr Chauhan

— Econ/Pol reasons for lack of money for vaccine research

—

(12)

The Second Sir Biren Mukherjee Tata Symposium

March 10-11, 2011

6 Dr G.P. Dhillon (contd)

Treatment Schedule

i) Primaquine sector do not clear blood smears — RII / RIII 25% in 30 cases

ii) High Risk Areas — Day-1 150 Chlo
4 Sng Primaquine

? After chemoprophylaxis — use Chloro/PQ full course

(7) Chevas Chivinus

- Erythrocyte Invasion
- Adherence (Functional binding domain)

(8) Sarala Subbha Rao — Vectors in India

An — 58 species — 10-15 — 6 spec
Karwar complex — An fl, An cul, An stp — An ma, Ad dun, An sundaic.

— Vectors and SAARC — cross boundaries
??

Oneroki 286 species/4 positive work

An. fluviatilis

Species S — An Phop I — Hyper endo

T U — Zoophop — Hypo endo

— Role of species in Malaria Transmission

— Biological characteristics of Primary Mal. Vect.

Breeding sites specificity | Anthropophilic index 0-8%

Biting activity (Peak biting time)

Vector Potential

— Drug Resistance (All vectors capable of transmitting resistance)

— Sibling species (diff gene pools)

differentiated by

— Anthropophilic index | Biting Activity | Peak Biting Time | Vector Potential | Sporozoite Rate | Resistance DDT | BHC | Malathion

— use Sibling species studies to

- plan control measures
- focus activity better

(9) N.J. Shetty

Genetic Control of Malaria — An. stephensi — susceptibility studies / parasites

Cytogenetics

— Genetic control — Studies on Mutants (inversions) — Studies on Chromosome

— Genetic sexing system

—

Nature Vol 389
4th Sept 1997

Sterile insect technology

The Second Six Borough Tele-Symposium

March 10, 11, 2004

(4) Dr Dhawan (contd)

Drugs Abandoned - Amodiaquin - Agranulocytosis / Hepatic necrosis
Fansidar — side effects

Ideal drug - Cheap
- widely available
- long half life
- effective schizonticide
- less toxic
- minimum drug interactions
(not mefloquin)

Chemoprophylaxis Problems
- lack of uniform guidelines for Physician
- Adverse effects
- Drug Resistance
- Immunity

NEJM, 1993

Compliance - - Difficult to adhere / Sustain
- bitter taste
- side effects
- 30-60%

Adverse Effects
Chloroquin - Retinopathy
Proguanil - Mouth ulcers
Mefloquin - Neuro Psychiatry
Doxycycline - Photosensitivity
Dapsone - Agranulocytosis

Drug Resistance — Chloroquin Resistance (India) — Multi drug resistance (other com...

Recommendations — ~~Sensitive~~ area: Chloroquin — Resistance area: Chloroquin + Proguan...

(5) Dr G.P. Dhillon — Scenario / Policy

) Malaria recorded 2-3 million / cases —

EMCP

EDPT — Active / Passive surveillance — Malaria Link Volunteers
Diagnosis by Malaria Microscopy — Supplying microscope
Treatment : Chloroquin and Sulphadoxine — Safer injectable Artemisinin

Selective Vector Control — INSecticides - DDT, Malathion
Chemical larvicide
Promotion of Personal protection method

Provision for biodegradable (Safer biolarvicides are used here)
Financial assistance for larvivorous fish hatcheries
Safety of medicated nets
Social marketing

- IEC for Community involvement
- Capacity Building
- Information System
- Strengthening operational aspect

Socio Epidemiology

Mehra

Poor infrastructure
Inadequate RS

Neeru Singh

Hydroelectric Project (High level Chloroquin Resist)
(Chloride change due to major dam)
Operational failures
Decline in insecticide coverage

Krishna

- ME/Professionals/Researchers not interested
- Drug Availability

Pedne

MNCs interes

Dhan

- Socio econ problems
- Movement of non-immune to malaria areas
- Multiple Regimens

GPD

- 2-3 million in Govt (Rest in PVT)
- MPW ↓
- IEC ??
- Peoples acceptance
- vector bionomics to community habits
- medic reports rather than MIS

Chauhan

Econ/Political Reasons why not researched

Socio-SR

Agricultural Practices
Deforestation
Socio-econ in workforce

cost of insecticide

(Shortfall/Inefficiency of ITMN use (not used is WHO project)

- Intersectoral collaboration
- UKP project Ker
- Visakh Steel Plant
- Population migration
- Urbanization/Industrial

Malaria Friendly Protagonists

Biomedical construed Professions/Researches

Drug companies

Private Practice oriented clinicians/

Construction industry.

Dev. projects & our HIA

Malaria Antagonists

Socio-epidemiologically oriented professionals Research

Public Health/Service oriented clinicians/Defence Services

Dev. projects & HIA

The Second Sir Dorabji Tata Symposium

March 10-11, 2001

3. Dr Neeru Singh MRC

Tribal / Forest - Malaria - MRC

- Neeyonjury → ecology changed due to H.E. Dams

— Objective

Peltern

Annual/Seasonal changes

Anti malarial measures

— Vector incrimination

- An culifacies - species C & D
- An. fluviatilis species S & T (Rice fields)
- Species C & D ? Vectors
  S & T

— Epid studies - 5 inaccessible - 2 months villages

— Pv Vs Pf

— neonatal parasitemia

— Pregnant women prevalence highest in II Trimester

Adverse Effects - Anemia, Abortion, 3% Still birth 4%, Neonatal deaths

1986-2000

1986 - DDT 2 rounds (1 gm/m²)

1. Drugs through MPW

1994. BHC 3 rounds

Drugs - MPW/School Teacher/Forest Guard

1997 Enhanced M.C.P

— EDPT

— Treated Bednets

— Bio control

Singh et al. Ann. Trop Med Parasitol - 95(1), 19-29

4. Malaria Chemoprophylaxis (SK Dhan)

Indian scenario - 2 million cases annually.

- upsurge of malaria since 1994 - outbreaks in northeast/western state
- ↑ incidence falciparum malaria
- Responsible for 40% of malaria deaths

Worsening of Scenario

- Resistance to antimalarials
  Vector Resis
  SE problem
  Movement of non-immune malaria o..

Chemoprophylaxis Drugs

- Tissue schizonticides
- Blood schizonticides

Control Strategy - Two major important components

- Treatment
- Chemoprophylaxis
- Avoidance of Mosquitoes (Risk to Dams)
  - Screened area
  - ITMN
  - Skin & clothes
  - insect Repellents

Chemoprophylaxis - control strategy is endemic area

- Prevent clinical malaria

Chloroquine | Proguanil | Dapsone | Pyrimethamine | Mefloquin | Doxycycline

Dr Kashyap (contd)

Clinical spectrum

- Vague mild illness
- Fever of any degree or pattern
- Any symptom pertaining to CNS/GIT/Resp System

Critical change in thinking

- Retinal ± Subconjunctival haemorrhages
- Anemia, hepatosplenomegaly. jaundice conjugated and unconjugated hyperbilirubinemia

Atypical Symptomatology

- Afebrile Malaria (Mehta SR et al JAPI 1996)
- Giddiness
- Tachypnoea and/or cough
- Neck rigidity — (exclude meningitis)
- Purpura and urticaria — DIC/or coagulopathy
- Pain abdomen
  ↓
  can be severe (acute pancreatitis)

Symptom complex

- Clinical profile of RI ± RII (PF)

- Acute Pulmonary oedema or ARDS (Anand.V, Mehta SR JAPI, 1994)
- Neuro signs / Symmetrical UMN — signs
- Malaria in Pregnancy
  - Maternal anemia
  - Abortion
  - Stillbirth
  - Intrauterine Growth Retardation
  - Low
- Abnormal Respiratory Patterns
- Anemia
  - common in children
  - G6PD deficient haemolysis
  - Dyserythropoiesis
  - Splenomegaly
  - Depletion of folate stores
  - Blackwater fever
- Fluid, electrolyte and acid base balance.
- Spontaneous bleeding and coagulopathy.
- Malarial Haemoglobinuria
- Clinical indicators of severe — poor prognosis
- HIV & Malaria

Taylor TE. Hofman IF Lancet 2000; 356 1046

Malaria in Differential D

1. Viral fever
2. Hepato-renal syndrome
3. Encephalopathy
4. ARDS
5. Anaemia
6. Gm-ve septicaemia — Atypical Malaria
7. Viral Hepatitis

Hypoglycemia in Malaria

- often recurrent
- more in children and pregnant women
- with severe disease
- Quinine > Quinidine RI.

1000 pts
18 adults
9 children

Good Study

Severe malaria

	Ad.	Ch
• Duration	5-7 da.	1-2
• Cough	uncommon	common
• Acidosis	common	common
• Hypogly.	common	

May other features

Neurological Sequelae

- Post malaria neurological Syndrome — Cerebellar (complete recovery 4-6 wks)

Conclusions?:

The Second Sir Dorabji Tata Symposium

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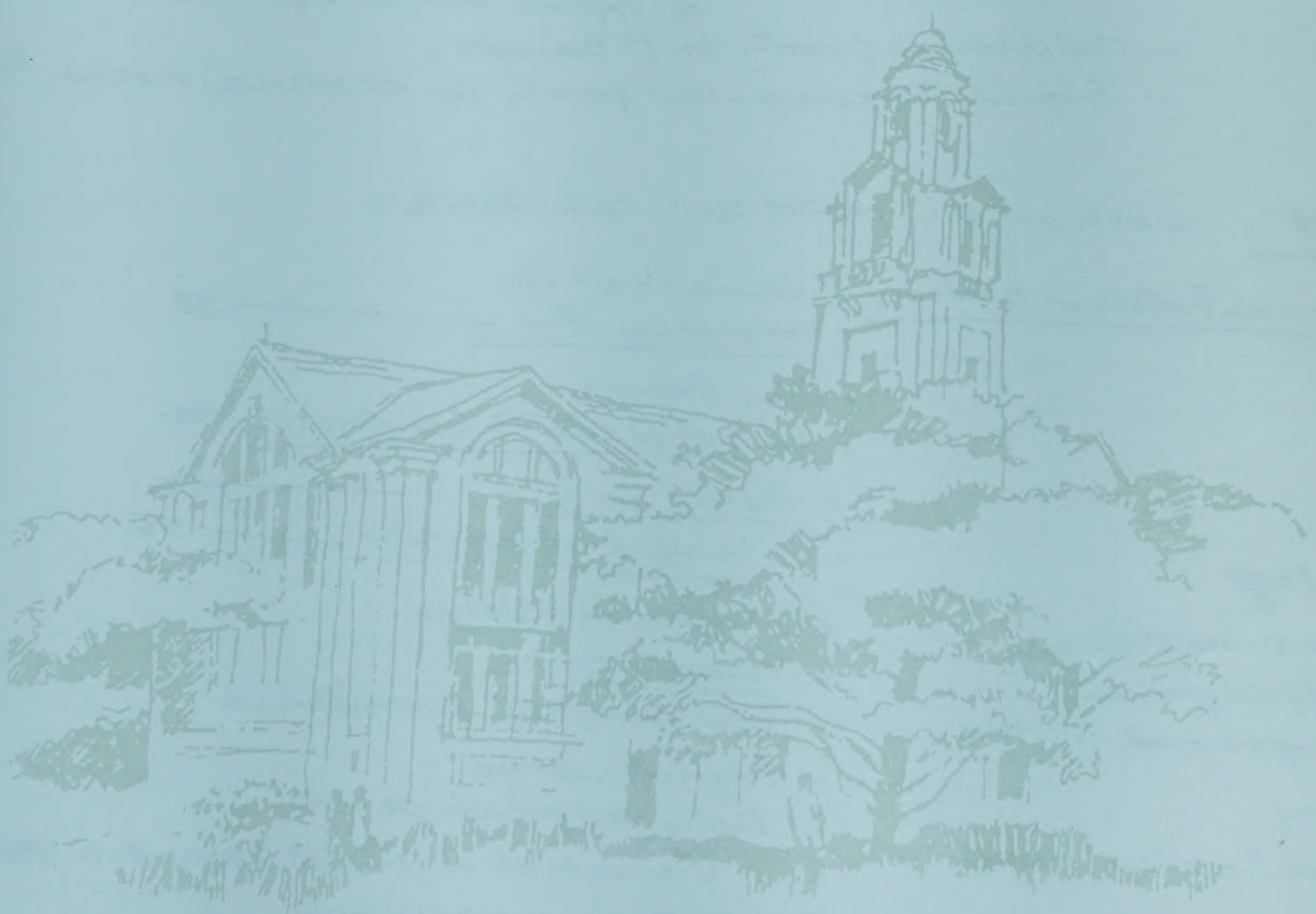

10/3 Malaria Symposium

1. Dr Padmanabhan

1. Genetics — Vaccine not in forseeable future
2. Drug Resistance —— Resistant to chloroquine — Can it be Reverse. Artemisinin ↑
3. Vaccine development - Difficulties
   ↓
   1) Antigenic diversity / immune evasion
   2) Lack of good in vitro correlates of protection
   3) Difficulties of large scale production of candidate vaccine
   4) Lack of proven delivery systems and adjuvants
   5) Difficulty and expense of human trials (No MNC/Drug companies
      ↓
      Too many stages to tackle — Sporozoite, Merozoite, Gametocyte } combination Required
      (Single antigens will not produce results)

- Malaria-Vaccine Candidates

DNA vaccines - major players / boosted by protein vaccine
(we need both humoral & cell mediated immunity)

- Genomics to Vaccines — Hoffman et al Nature Medicine 4 1351-1357

(Mega approach leading to vaccinome)

2. Gen. Krishnan

- Gen Montgomery — More soldiers died of Typhus than of Japanese bullets
- Malaria - low priority in M Educ
- Tropical Diseases - low priority in academic / research today

a) Dr Kashyap — Warhurst DC NEJM 2001; 344: 299-302

Malaria - Problem Statement — Causes high M.
- empirical Rx —— SE
- Drug Resist.
- Uncomplicate → Complicate — SE
- Delay in Δ —— SE
- Lack of infrastructure —— SE
- Vector Resistance
- Missed Δ
  Low peripheral parasitemia
- Pregnant women
- Early postpartum period
  Diagne N et al NEJM 2000: 343: 598-603
- Children / anemic patients
- Visitors - non endemic areas — Travellers / Tourists / Soldiers

- Extremes of age
- Pregnancy - primigravidae
  gestation / postpartum
- Immunosuppressed - HIV / AIDS
- Splenectomy
- Non immune travellers / migrants